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**Title of article**

The influence of capillarization on satellite cell pool expansion and activation following exercise-induced muscle damage in healthy young men

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**Conflict of Interest**

There are no conflict of interests.

## **ABSTRACT:**

Factors that determine the skeletal muscle satellite cell (SC) response remain incompletely understood. It is known, however, that SC activation status is closely related to the anatomical relationship between SC and muscle capillaries. We investigated the impact of muscle fibre capillarization on the expansion and activation status of SC following a muscle damaging exercise protocol in healthy young men. Twenty-nine young men ( $21 \pm 0.5$  yrs) performed 300 unilateral eccentric contractions ( $180 \text{ deg} \cdot \text{s}^{-1}$ ) of the knee extensors. Percutaneous muscle biopsies from the vastus lateralis and blood samples from the antecubital vein were taken prior to (Pre) and at 6h, 24h, 72h and 96h of post-exercise recovery. A comparison was made between subjects who had a relative low mixed muscle capillary-to-fibre perimeter exchange index (CFPE; Low group) and high mixed muscle CFPE index (High group) at baseline. Type I and type II muscle fibre size, myonuclear content, capillarization, and SC response were determined via immunohistochemistry. Overall, there was a significant correlation ( $r = 0.39$ ;  $p < 0.05$ ) between the expansion of SC content (change in total Pax7<sup>+</sup> cells/100 myofibre) 24h following eccentric exercise and mixed muscle CFPE index. There was a greater increase in activated SC (MyoD<sup>+</sup>/Pax7<sup>+</sup> cells) in the High as compared to the Low CFPE group 72h following eccentric exercise ( $p < 0.05$ ). The current study provides further evidence that muscle fibre capillarization may play an important role in the activation and expansion of the SC pool during the process of skeletal muscle repair.

## INTRODUCTION:

Skeletal muscle satellite cells (SC) are indispensable for muscle regeneration and repair following injury (Lepper *et al.*, 2011; McCarthy *et al.*, 2011; Sambasivan *et al.*, 2011). In response to a physiological cue (e.g. exercise), SC activate, proliferate and differentiate donating nuclei to existing muscle fibres to aid in repair/adaptation or return to a state of quiescence to replenish the basal SC pool (Bentzinger *et al.*, 2012; Yin *et al.*, 2013). The process of SC activation through terminal differentiation is orchestrated by a transcriptional network, known as the myogenic regulatory factors (MRFs), and is collectively referred to as the myogenic program. Expansion of the SC pool following a single bout of exercise or muscle fibre contraction-induced damage has been well characterized in humans (McKay *et al.*, 2008; McKay *et al.*, 2009; McKay *et al.*, 2012; McKay *et al.*, 2013; Bellamy *et al.*, 2014; Nederveen *et al.*, 2017) with appreciable expansion occurring by 24h and peaking 72h post-stimulus (Snijders *et al.*, 2015).

A number of cytokines and growth factors including, but not limited to, interleukin-6 (IL-6), insulin-like growth factor-1 (IGF-1), myostatin and hepatocyte growth factor (HGF) are known regulators of SC progression through the myogenic program (McKay *et al.*, 2008; O'Reilly *et al.*, 2008; McKay *et al.*, 2009). Many of these factors are produced by skeletal muscle in its function as an 'endocrine organ' (Steensberg *et al.*, 2000; Pedersen & Febbraio, 2008), or by other organs, tissues or cells (Velloso, 2008) and then delivered to the SC niche via the vasculature. Therefore, delivery of these factors to the SC niche may be a requirement of the myogenic response. Indeed, the importance of extrinsic factors in regulating SC function has been demonstrated using parabiotic pairings of old and young rodents (Conboy *et al.*, 2005; Brack & Rando, 2007).

Muscle capillaries function as the delivery mechanism for oxygen, fuel, cytokines and growth factors that may regulate SC, but may also act as an important modulator of the SC response. We and others have reported an anatomical relationship between muscle SC and the microvasculature, with activated SC situated geographically closer to capillaries than quiescent SC (Christov *et al.*, 2007; Nederveen *et al.*, 2016; Nederveen *et al.*, 2017). Consequently, it has been proposed that SC content (Emslie-Smith & Engel, 1990; Christov *et al.*, 2007) and/or SC activation status (Chazaud *et al.*, 2003; Christov *et al.*, 2007; Nederveen *et al.*, 2016) may be related to the extent of muscle fibre capillarization as a result of exposure of SC to circulating factors or direct communication between endothelial cells and SC during muscle repair (Chazaud *et al.*, 2003; Ochoa *et al.*, 2007; Joannis *et al.*, 2017). However, to what extent the muscle fibre microvascular bed may dictate the acute muscle SC response during muscle repair in humans remains unknown. Therefore, in the present study, we assessed the expansion and activation status of the SC pool following a single bout of exercise-induced muscle fibre damage in a group of healthy young men with varying degrees of muscle fibre capillarization. We hypothesized that individuals with a greater degree of muscle fibre capillarization would demonstrate a more rapid and pronounced SC response following a single bout of eccentric exercise.

## **METHODS**

***Participants.*** Twenty nine healthy young men (YM:  $22 \pm 0.5$  yr; mean  $\pm$  SEM) were recruited to participate in this study. Exclusion criteria included smoking, diabetes, the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and/or statins, and a history of respiratory disease and/or any major orthopaedic disability. Subjects were told to refrain from exercising throughout the duration of the study, and refrain from the use of NSAIDs (Mackey *et al.*, 2016). The study was approved by the Hamilton Health Sciences Integrated Research Ethics Board, and conformed to

the guidelines outlined in the Declaration of Helsinki. Participants gave their informed written consent prior to inclusion in the study.

***VO<sub>2peak</sub> test and anthropometric measurements.*** During an initial visit to the laboratory participants performed a VO<sub>2peak</sub> test on a cycle ergometer (model: H-300-R Lode; Lode B.V., Groningen, The Netherlands) and had anthropometric measurements recorded. The VO<sub>2peak</sub> test consisted of load-less pedaling for one minute, followed by a step-wise increase to 50 watts (W) for two minutes. After the increase to 50 watts, work rate was increased by 30 W/min until the participant reached volitional fatigue (determined by the inability of the participant to maintain a minimum cadence of 60 RPM). Gas exchange was collected throughout the test using a metabolic cart (Moxus, AEI Technologies, Pittsburgh, PA) and VO<sub>2peak</sub> was calculated using the highest 30 second average VO<sub>2</sub> during the final stage of the ramp protocol. Work rate (WR) was collected continuously throughout the test and peak aerobic power (WR<sub>peak</sub>) was calculated using the average WR from the last 30 seconds of the test.

***Muscle biopsy sampling.*** Percutaneous needle biopsies were taken, after an (~10h) overnight fast (Pre), from the mid-portion of the *vastus lateralis* under local anesthetic using a 5 mm Bergstrom needle adapted for manual suction. Subjects had not participated in any physical activity for at least 96 hours before muscle biopsy collection prior to the single bout of eccentric exercise. The muscle biopsy procedure was repeated at 6h, and in the fasted condition (~10h) 24h, 72h and 96h of post-exercise recovery.. Incisions for the repeated muscle biopsy sampling were spaced approximately 3 cm apart to minimize any effect of the previous biopsy. Upon excision, muscle samples were immediately mounted in optimal cutting temperature (OCT) compound, frozen in liquid nitrogen-cooled isopentane, while another part was directly frozen in liquid nitrogen, and stored at -80° C until further analyses.

**Blood sampling.** Blood samples were obtained from the antecubital vein immediately prior the muscle biopsy sampling procedure before and after 6h, 24h, 72h and 96h of the single bout of eccentric exercise. Blood (~10 mL) samples were collected in EDTA containing tubes and centrifuged at 1500 rpm for 10 min at 4 °C. Aliquots of plasma were frozen in liquid nitrogen and stored at -80°C. Plasma samples were analyzed for IL-6 protein and creatine kinase activity using commercially available Enzyme-Linked ImmunoSorbant Assay (ELISA) (R & D Systems, Inc., USA) and activity assay kits (Abcam Inc., Canada), respectively, following the manufacturer's instructions. Statistics were performed on the raw values, and expressed as a percentage change from baseline.

**Eccentric Muscle Damage Protocol.** Maximal isokinetic unilateral muscle-lengthening contractions of the quadriceps were performed using the Biodex dynamometer (Biodex-System 3, Biodex Medical Systems, Inc., USA) at 180 deg s<sup>-1</sup>. For each subject, one leg was selected randomly to perform the exercise protocol described below. Movement at the shoulders, hips and thigh were restrained with straps in order to isolate the knee extensors during the protocol. Immediately prior to the intervention, subjects underwent a brief familiarization with the equipment, involving 5–10 submaximal lengthening contractions of the leg to be exercised. Subjects were required to perform 30 sets of 10 maximal knee extensions with 1 min rest between sets, for a total of 300 lengthening contractions. During each set, investigators provided verbal encouragement for the subjects to complete and exert maximal force during each contraction. This protocol has been previously shown to induce a significant level of skeletal muscle damage (Beaton *et al.*, 2002).

**Immunofluorescence.** Muscle cross sections (7µm) were prepared from unfixed OCT embedded samples, allowed to air dry for 30 minutes and stored at -80°C. Samples were stained with

appropriate primary and secondary antibodies against specific antigens, found in Table 1, as previously described (Nederveen *et al.*, 2016). Nuclei were labelled with DAPI (4',6-diamidino-2-phenylindole) (1:20000, Sigma-Aldrich, Oakville, ON, Canada), prior to cover slipping with fluorescent mounting media (DAKO, Burlington, ON, Canada). The staining procedures were verified using negative controls, in order to ensure appropriate specificity of staining. Slides were viewed with the Nikon Eclipse *Ti* Microscope (Nikon Instruments, Inc. USA), equipped with a high-resolution Photometrics CoolSNAP HQ2 fluorescent camera (Nikon Instruments, Melville, NY, USA). Images were captured and analyzed using the Nikon NIS Elements AR 3.2 software (Nikon Instruments, Inc., USA). All images were obtained with the 20x objective, and  $\geq 200$  muscle fibres/subject/time point were included in the analyses for SC content/activation status (i.e., Pax7<sup>+</sup>/MyoD<sup>-</sup> or Pax7<sup>+</sup>/MyoD<sup>+</sup>), and fibre cross sectional area (CSA), and perimeter. The activation status of SCs was determined via the colocalization of Pax7<sup>+</sup> and DAPI (Pax7<sup>+</sup>/MyoD<sup>-</sup>) and/or the co-localization of Pax7, MyoD and DAPI (i.e., Pax7<sup>+</sup>/MyoD<sup>+</sup>). Cell membranes were labelled with Peroxidase conjugated Wheat Germ Agglutinin (WGA) (1  $\mu$ g/mL, Vector PL-1026, Burlington, ON, Canada) and realized with a substrate kit (Vector, SK-4700, Burlington, ON, Canada) as per manufacturer's instructions. Slides were blinded for both group and time point. The quantification of muscle fibre capillaries was performed on 50 muscle fibres/subject/time point (Porter *et al.*, 2002). Based on the work of Hepple *et al.* (Hepple, 1997; Hepple & Mathieu-Costello, 2001), quantification of; i) capillary contacts (CC; the number of capillaries around a fibre), ii) the capillary-to-fibre ratio on an individual fibre basis (C/Fi), iii) the number of fibres sharing each capillary (i.e., the sharing factor) and iv) the capillary density (CD) was performed. The CD was calculated by using the cross sectional area ( $\mu$ m<sup>2</sup>) as the reference space. The capillary-to-fibre perimeter exchange index (CFPE) was calculated as an



estimate of the capillary-to-fibre surface area (Hepple, 1997). The SC-to-capillary distance measurements were performed on all SC that were enclosed by other muscle fibres, and has been described previously (Nederveen *et al.*, 2016). All immunofluorescent analysis were completed in a blinded fashion.

**RNA Isolation.** RNA was isolated from 15–25 mg of muscle tissue using the Trizol/RNeasy method. All samples were homogenized with 1 mL of Trizol Reagent (Life Technologies, Burlington, ON, Canada), in Lysing Maxtrix D tubes (MP Biomedicals, Solon, OH, USA), with the FastPrep-24 Tissue and Cell Homogenizer (MP Biomedicals, Solon, OH, USA) for a duration of 40 sec at a setting of 6 m/sec. Following a five minute room temperature incubation, homogenized samples were stored at -80°C for one month until further processing. After thawing on ice, 200 ml of chloroform (Sigma-Aldrich, Oakville, ON, Canada) was added to each sample, mixed vigorously for 15 sec, incubated at RT for 5 min, and spun at 12000 g for 10 min at 4°C. The RNA (aqueous) phase was purified using the E.Z.N.A. Total RNA Kit 1 (Omega Bio-Tek, Norcross, GA, USA) as per manufacturer's instructions. RNA concentration (ng/ml) and purity (260/280) was determined with the Nano-Drop 1000 Spectrophotometer (Thermo Fisher Scientific, Rockville, MD, USA).

**Reverse Transcription.** Samples were reverse transcribed using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) in 20 µl reaction volumes, as per manufacturer's instructions, using an Eppendorf Mastercycler epGradient Thermal Cycler (Eppendorf, Mississauga, ON, Canada) to obtain cDNA for gene expression analysis.

**Quantitative RT-PCR.** All quantitative RTPCR reactions were run in duplicate in 25 µl volumes containing RT Sybr Green qPCR Master Mix (Qiagen Sciences, Valencia, CA, USA), prepared

with the epMotion 5075 Eppendorf automated pipetting system (Eppendorf, Mississauga, ON, Canada), and carried out using an Eppendorf Realplex2 Master Cycler epgradient (Eppendorf, Mississauga, ON, Canada). Primers are listed in Supplementary Table 1 and were re-suspended in 1X TE buffer (10mM Tris–HCl and 0.11 mM EDTA) and stored at –20°C prior to use.

Messenger RNA expression was calculated using the  $2^{-\Delta\Delta C_t}$  method, and fold changes from baseline were calculated using the  $\Delta\Delta C_t$  method (Livak & Schmittgen, 2001). Briefly, Ct values were first normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Supplementary Table 1). Ct values normalized to GAPDH were expressed as delta-Cts ( $\Delta C_t$ ).  $\Delta C_t$  values were then normalized to Pre values, expressed as delta-delta Cts ( $\Delta\Delta C_t$ ). Values were then transformed out of the logarithmic scale using the formula: fold change =  $2^{-\Delta\Delta C_t}$  (Livak & Schmittgen, 2001). Thus, mRNA values are expressed as a fold change from Pre (mean  $\pm$  sem). GAPDH expression was not different from Pre at any of the post-intervention time-points.

**Statistical analysis.** Statistical analysis was performed using Sigma Stat 3.1.0 analysis software (Systat Software, Chicago, IL, USA). *Stratification of individuals based on capillarization.* To assess the impact of muscle fibre capillarization on the muscle SC response following a single bout of eccentric exercise and subsequent muscle damage, participants were assigned into one of two groups (n = 10 per group) based on mixed muscle fibre capillarization (corrected for capillary sharing factor and muscle fibre perimeter, also known as capillary-to-fibre perimeter exchange (CFPE) index) for non-correlative statistical analysis. This resulted in a group with a relatively low (Low; CFPE:  $5.2 \pm 0.5$  capillaries  $\cdot 1000 \mu\text{m}^{-1}$ ) and relatively high (High; CFPE:  $7.6 \pm 1.0$  capillaries  $\cdot 1000 \mu\text{m}^{-1}$ ) mixed muscle fibre CFPE index. Stratification of participants resulted in a ‘middle’ group (n = 9) who were not used in non-correlative statistical analysis,

with the intent to create a clear separation between the Low and High group. *Baseline comparisons.* Comparisons of participant demographics between the High CPFE and the Low CPFE groups are found in Table 2, and were performed via a Student's *t* test. Baseline comparisons of muscle fibre type specific characteristics between the High CPFE and the Low CPFE group were performed using a two-way ANOVA (group x fibre type). *Response to eccentric contractions.* One-way repeated measures ANOVA were performed separately for each of the 'Overall' group, for the High CPFE group and for the Low CPFE group, with time (Pre, 6h, 24h and 72h and 96h) as a within group factor. In these one-way repeated measures ANOVA design for the acute response, post-exercise time points were only compared with baseline (Pre) and Bonferonni corrections were applied to account for multiple comparisons. These tests were performed to assess the following; the acute change in SC activity status (i.e., Pax7+/MyoD+ cells); the acute change in SC content (i.e., mixed muscle, type I and/or type II Pax7+ cells); the acute change in plasma IL-6 content; the acute change in plasma creatine kinase activity; the acute change in quadriceps muscle force production and the acute change in MRF mRNA expression, following the bout of eccentric exercise induced muscle damage. For correlations, Pearson's correlation analyses were performed where appropriate between indices of muscle fibre capillarization and the SC response following eccentric exercise. Statistical significance was accepted at  $p < 0.05$ . All results were presented as means  $\pm$  standard error of the mean (SEM).

## **RESULTS**

### **Subject characteristics**

*Overall:* Complete subject characteristics are reported in Table 2.

*Low vs. High CFPE group:* There were no differences in age or height between the groups (Table 2). There was a significant difference in bodyweight ( $p < 0.05$ , Table 2) and a trend for BMI ( $p = 0.06$ , Table 2) between the groups. Both the  $\text{VO}_{2\text{max}}$  (mL/kg/min) and  $W_{\text{peak}}$  (W) was significantly greater in the High as compared to the Low group ( $p < 0.05$ ; Table 2). There was no significant difference in force production prior to single bout eccentric exercise in the High ( $272.6 \pm 8.8 \text{ N}\cdot\text{m}$ ) compared to the Low ( $314.4 \pm 13.3 \text{ N}\cdot\text{m}$ ) group (Table 2).

### **Indices of muscle damage following repeated eccentric contractions.**

*Overall:* Following eccentric contractions, force production ( $\text{N}\cdot\text{m}$ ) was significantly reduced at 6h ( $253 \pm 15 \text{ N}\cdot\text{m}$ ), 24h ( $233 \pm 16 \text{ N}\cdot\text{m}$ ), 72h ( $261 \pm 18 \text{ N}\cdot\text{m}$ ) and 96h ( $270 \pm 17 \text{ N}\cdot\text{m}$ ), as compared to Pre ( $319 \pm 14 \text{ N}\cdot\text{m}$ ) ( $p < 0.05$ ; Supplementary Fig 1A). Following the eccentric contraction protocol, plasma creatine kinase activity was significantly increased at 24h ( $103.7 \pm 8.2 \text{ mU/mL}$ ,  $p < 0.05$ ; Supplementary Fig 1C) compared to Pre ( $75.4 \pm 6.1 \text{ mU/mL}$ ), and returned back to baseline at 72h ( $76.3 \pm 6.7 \text{ mU/mL}$ ) and 96h ( $79.1 \pm 6.6 \text{ mU/mL}$ )

*High CFPE vs Low CFPE group:* In the High group, force production was significantly reduced at 6h ( $211 \pm 15 \text{ N}\cdot\text{m}$ ), 24h ( $196 \pm 17 \text{ N}\cdot\text{m}$ ), 72h ( $230 \pm 21 \text{ N}\cdot\text{m}$ ) as compared to Pre ( $272 \pm 15 \text{ N}\cdot\text{m}$ ) and was back at baseline levels again at 96h ( $241 \pm 23 \text{ N}\cdot\text{m}$ ) ( $p < 0.05$ ; Supplementary Fig 1B). In the Low group, force production was significantly reduced at 6h ( $248 \pm 29 \text{ N}\cdot\text{m}$ ), 24h ( $215 \pm 34 \text{ N}\cdot\text{m}$ ), 72h ( $242 \pm 37 \text{ N}\cdot\text{m}$ ) and 96h ( $257 \pm 32 \text{ N}\cdot\text{m}$ ) as compared to Pre ( $314 \pm 23 \text{ N}\cdot\text{m}$ ) ( $p < 0.05$ ; Supplementary Fig 1B). In the High group, plasma creatine kinase activity was significantly increased 24h ( $93.3 \pm 13.8 \text{ mU/mL}$ ,  $p < 0.05$ ) following eccentric exercise, but was not significantly different at 72h ( $56.0 \pm 5.4 \text{ mU/mL}$ ) and 96h ( $66.4 \pm 8.6 \text{ mU/mL}$ ) as compared to Pre ( $60.3 \pm 8.4 \text{ mU/mL}$ ) ( $p < 0.05$ ; Supplementary Fig 1D). In the Low group, plasma creatine

kinase activity was significantly increased 24h ( $107.9 \pm 10.7$  mU/mL,  $p < 0.05$ ) following eccentric exercise, but was not significantly different at 72h ( $92.9 \pm 12.4$  mU/mL) and 96h ( $91.1 \pm 14.3$  mU/mL) as compared to Pre ( $88.4 \pm 11.2$  mU/mL) ( $p < 0.05$ ; Supplementary Fig 1D). Prior to the intervention, there were no significant differences in creatine kinase activity in the High compared to the Low group; there were no differences in creatine kinase activity changes following eccentric exercise.

### **Skeletal muscle fibre characteristics**

*Overall:* Muscle fibre CSA was significantly greater in type II ( $7500 \pm 355$   $\mu\text{m}^2$ ) compared to type I fibres ( $6326 \pm 205$   $\mu\text{m}^2$ ,  $p < 0.05$ ). Muscle fibre perimeter was significantly greater in type II ( $326 \pm 6$   $\mu\text{m}$ ) compared to type I fibres ( $306 \pm 5$   $\mu\text{m}$ ,  $p < 0.05$ ). The number of myonuclei per fibre was not different between type II as compared to type I ( $3.7 \pm 0.2$  vs  $3.6 \pm 0.2$  myonuclei/fibre, respectively). Myonuclear domain size was significantly greater in type II as compared to type I muscle fibres ( $2019 \pm 88$  vs  $1805 \pm 53$   $\mu\text{m}^2$ ,  $p < 0.05$ , respectively). Muscle C/Fi ( $2.08 \pm 0.1$  vs.  $1.94 \pm 0.1$  capillaries per fibre), CFPE index ( $6.86 \pm 0.2$  vs.  $6.03 \pm 0.23$  capillaries per fibre  $\cdot 1000^{-1}$ , and CD ( $655 \pm 26$  vs.  $478 \pm 29$  capillaries/  $\text{mm}^2$ ) was significantly greater in type I compared to type II muscle fibres, respectively ( $p < 0.05$ ). SC distance to nearest capillary was significantly greater in type II compared to type I muscle fibres ( $15.8 \pm 0.7$  vs  $13.9 \pm 0.7$   $\mu\text{m}$ , respectively,  $p < 0.05$ ) at baseline (Pre). Total Pax7<sup>+</sup> cell distance to nearest capillary at baseline was negatively correlated to mixed muscle CFPE index ( $r = -0.49$ ,  $p < 0.05$ ) across all participants. Type II muscle fibre associated SC distance to nearest capillary at baseline was negatively correlated to type II fibre CFPE index ( $r = -0.51$ ,  $p < 0.05$ ; Figure 4D).

*High CFPE vs Low CFPE group:* Type II muscle fibre CSA and perimeter were significantly greater compared with type I muscle fibre in both groups, with no difference between group

( $p < 0.05$ ; Table 3). Interestingly, the proportion of type I muscle fibres was significantly higher in the High group compared with the Low group ( $p < 0.05$ , Table 3). Both type I and type II muscle fibre C/Fi, CFPE index, and CC were significantly higher in the High as compared to the Low group ( $p < 0.05$ ; Table 3). There were no significant differences ( $p > 0.05$ ) in type I or type II myonuclei per fibre and/or myonuclear domain size between groups.

SC distance to nearest capillary across both type I and type II was significantly lower in the High group as compared to the Low group ( $p < 0.05$ , Table 3).

### **Mixed muscle SC response**

*Overall:* Following the eccentric contraction protocol, total mixed muscle Pax7<sup>+</sup> cells/100 myofibre tended to increase significantly at 6h ( $14.4 \pm 1.1$  cells/100 myofibre ( $p = 0.056$ ), increased significantly at 24h ( $14.9 \pm 1.1$  cells/100 myofibre;  $p < 0.05$ ) and 72h ( $15.8 \pm 1.0$  cells/100 myofibre;  $p < 0.05$ ) compared to Pre ( $11.8 \pm 0.7$  cells/100 myofibre, Supplementary Table 2). The change in total mixed muscle Pax7<sup>+</sup> cells/100 myofibre between Pre and 24h ( $r = 0.39$ ,  $p < 0.05$ ; Figure 1G) was positively correlated to mixed muscle CFPE index across all participants. The activation status of the SC pool was assessed by colocalizing SC with MyoD before and after the eccentric contraction protocol. Mixed muscle Pax7<sup>+</sup>/MyoD<sup>+</sup> cells/100 myofibres were significantly elevated at 6h ( $1.8 \pm 0.3$  cells/100 myofibre,  $p < 0.05$ ), 24h ( $2.2 \pm 0.2$  cells/100 myofibre,  $p < 0.05$ ), 72h ( $1.9 \pm 0.4$  cells/100 myofibre,  $p < 0.05$ ) and 96h ( $1.1 \pm 0.2$  cells/100 myofibre,  $p < 0.05$ ) as compared to Pre ( $0.4 \pm 0.1$ ; Figure 2F). The change in total mixed muscle MyoD<sup>+</sup>/Pax7<sup>+</sup> cells/100 myofibre between Pre and 6h ( $r = 0.40$ ,  $p < 0.05$ ; Figure 1G) and Pre and 72 ( $r = 0.37$ ,  $p < 0.05$ ; Figure 1H) was positively correlated to mixed muscle CFPE index across all participants.

*High vs. Low CFPE Group:* Prior to the intervention, there were no differences in mixed muscle total Pax7<sup>+</sup> cells/100 myofibre ( $p>0.05$ ) between the High ( $11.0 \pm 1.2$  cells/100 myofibre) and the Low ( $11.9 \pm 1.3$  cells/100 myofibre) group. Compared to baseline, total mixed muscle Pax7<sup>+</sup> cells/100 fibre were significantly increased at 6h ( $p<0.05$ ); 24h ( $p<0.05$ ) and 72h ( $p<0.05$ ) after the single bout of eccentric exercise in the High group (Figure 1F, Supplementary Table 3). In contrast, total mixed muscle Pax7<sup>+</sup> cells/100 myofibre was only significantly increased at 72h ( $p<0.05$ ) during post-exercise recovery in the Low group compared to baseline (Figure 1F, Supplementary Table 3). There was a significantly greater increase in mixed muscle total Pax7<sup>+</sup> cells/100 myofibres from Pre to 6h ( $p<0.05$ ); Pre to 24h ( $p<0.05$ ) and a trend for Pre to 72h ( $p=0.052$ ) following eccentric contractions in the High compared with the Low Group.

Prior to the intervention, there were no differences in total MyoD<sup>+</sup>/Pax7<sup>+</sup> cells/100 myofibre in mixed muscle ( $p>0.05$ ) between the High ( $0.3 \pm 0.2$  cells/100 myofibre) and the Low ( $0.2 \pm 0.1$  cells/100 myofibre) groups. Mixed muscle Pax7<sup>+</sup>/MyoD<sup>+</sup> cells/100 myofibres were significantly higher in the High group at 6h ( $p<0.05$ ), 24h ( $p<0.05$ ), 72h ( $p<0.05$ ), and 96h ( $p<0.05$ ) as compared to Pre (Figure 2F, Supplementary Table 3). In the Low group, Pax7<sup>+</sup>/MyoD<sup>+</sup> cells/100 myofibres in mixed muscle were only significantly elevated at 24h ( $p<0.05$ ) as compared to Pre (Figure 2F, Supplementary Table 3). In comparing the Low and the High mixed muscle SC activation (Pax7<sup>+</sup>/MyoD<sup>+</sup> cells) response to eccentric exercise, we observed that there was a significantly greater increase in the number of mixed muscle Pax7<sup>+</sup>/MyoD<sup>+</sup> cells/100 myofibre from Pre to 6h, and from Pre to 72h post-exercise recovery in the High as compared to the Low group ( $p<0.05$ , Figure 2F).

### **Type I and type II muscle fibre SC response**

*Overall:* Prior to the intervention, there was no significant difference between type I-associated ( $11.5 \pm 0.9$  cells/100 myofibre) and type II-associated Pax7<sup>+</sup> cells/100 myofibres ( $11.8 \pm 1.0$  cells/100 myofibre) across all participants ( $p > 0.05$ , Supplementary Table 2). Type I-associated Pax7<sup>+</sup> cells/100 myofibres remained unchanged at 6h, 24h, 96h and trended towards a significant increase at 72h ( $p = 0.09$ ), as compared to Pre (Supplementary Table 2).

Type II-associated Pax7<sup>+</sup> cells/100 myofibres remained unchanged at 6h and 24h but increased significantly at 72h ( $p < 0.05$ ), returning to basal levels at 96h as compared to Pre (Figure 3A).

The change in type II-associated Pax7<sup>+</sup> cells/100 myofibre between Pre and 6h ( $r = 0.45$ ,  $p < 0.05$ ; Figure 3B) and Pre and 24h ( $r = 0.42$ ,  $p < 0.05$ ; Figure 3C) following eccentric exercise was positively correlated with type II CFPE index across all participants.

The change in type II associated SC Pax7<sup>+</sup> cells/100 myofibre from Pre to 24h ( $r = -0.37$ ,  $p < 0.05$ ; Figure 4B) following eccentric exercise was negatively correlated to type II SC distance to nearest capillary at baseline across all participants. There were no relationships between type I associated SC and type I SC distance to nearest capillary at baseline across all participants.

*High vs. Low CFPE Group:* Prior to the intervention, there were no differences in type I-associated Pax7<sup>+</sup> cells/100 myofibre or type II-associated Pax7<sup>+</sup> cells/100 myofibre between Low and High groups. ( $p > 0.05$ ; Table 3). Type I Pax7<sup>+</sup> cells/100 myofibre was not significantly changed at 6h, 24h, 72h, or 96h as following eccentric contractions compared to Pre in the High ( $p > 0.05$ ) or Low group ( $p > 0.05$ , Supplementary Table 3). In comparing the Low group to the High group following eccentric exercise, there were no differences between Type I Pax7<sup>+</sup>/100 myofibres between Pre and any post-exercise time point. Type II Pax7<sup>+</sup> cells/100 myofibre were



significantly increased at 6h ( $p<0.05$ ) 24h ( $p<0.05$ ) and 72h ( $p<0.05$ ) following eccentric contractions in the High group, as compared to Pre (Figure 3A, Supplementary Table 3).

In the Low group, Type II Pax7<sup>+</sup> cells/100 fibre was only significantly elevated at 72h ( $p<0.05$ ), as compared to Pre (Figure 3A, Supplementary Table 3). In comparing the Low and the High muscle fibre type specific SC response to eccentric exercise, we observed that there was a greater change in the number of Type II Pax7<sup>+</sup> cells/100 myofibres from Pre to 6h, and from Pre to 24h post-exercise in the High group as compared to the Low ( $p<0.05$ , Figure 3A, Supplementary Table 3).

### **SC distance to nearest capillary response following eccentric**

*Overall:* SC distance to nearest capillary in mixed muscle fibres and/or type I/II-associated SC did not change in response to the single bout of eccentric exercise.

*High vs. Low CFPE Group:* Type I SC distance to nearest capillary did not change ( $p>0.05$ ) following eccentric contractions in either the High (Pre:  $12.2 \pm 0.8$  ; 6h:  $13.0 \pm 0.9$ ; 24h:  $12.4 \pm 0.8$ ; 72h:  $12.6 \pm 0.7$ ; 96h:  $11.9 \pm 1.2$   $\mu\text{m}$ ) or the Low group (Pre:  $13.9 \pm 0.9$ ; 6h:  $12.1 \pm 0.7$ ; 24h:  $17.0 \pm 1.1$ ; 72h:  $17.5 \pm 1.8$ ; 96h:  $15.0 \pm 0.9$   $\mu\text{m}$ ) as compared to baseline values. Type II SC distance to nearest capillary did not change ( $p>0.05$ ) following eccentric contractions in either the High (Pre:  $13.3 \pm 0.8$  ; 6h:  $13.1 \pm 1.3$ ; 24h:  $15.1 \pm 1.2$ ; 72h:  $16.0 \pm 1.0$ ; 96h:  $12.5 \pm 1.3$   $\mu\text{m}$ ) or the Low group (Pre:  $17.3 \pm 0.9$ ; 6h:  $17.0 \pm 1.0$ ; 24h:  $16.9 \pm 0.8$ ; 72h:  $20.0 \pm 1.5$ ; 96h:  $16.8 \pm 1.1$   $\mu\text{m}$ ) as compared to baseline values.

### **Myogenic regulatory factor response**

*Overall:* MyoD, MRF4 and Myogenin mRNA expression were significantly increased at 6h (2.2-, 1.8- and 4.4-fold change, respectively), 24h (1.4-, 2.1- and 4.0-fold change, respectively), 72h (1.6-, 1.6-, and 2.0-fold change, respectively) and 96h (1.4-, 2.3- and 1.9-fold change,

respectively) after the single bout of eccentric exercise. Myf5 mRNA expression was only significantly increased at 24h (1.6-fold change), 72h (1.6-fold change) and 96h (1.9-fold change) following exercise.

*High vs. Low CFPE Group:* In comparing the Low and the High myogenic gene mRNA expression in response to eccentric exercise, we observed that there was a greater change in MyoD mRNA expression from Pre to 72h in the High group as compared to the Low group ( $p < 0.05$ , Supplementary Table 4). We also observed that there was a trend for a smaller increase in Myogenin mRNA gene expression from Pre to 24h, in the High group as compared to the Low ( $p = 0.055$ ).

### **Cytokine response to repeated eccentric contractions**

*Overall:* Plasma IL-6 concentrations were significantly increased at 6h ( $2.2 \pm 0.2$  pg/mL,  $p < 0.05$ , Supplementary Figure 1E) and 24h ( $1.6 \pm 0.1$  pg/mL,  $p < 0.05$ ) but at 72h ( $1.2 \pm 0.1$  pg/mL) was not different anymore from Pre ( $1.1 \pm 0.1$  pg/mL). The change in plasma IL-6 between Pre and 6h ( $r = 0.42$ ,  $p < 0.05$ , Supplementary Figure 2A), as well as Pre and 72h ( $r = -0.42$ ,  $p < 0.05$ ) following eccentric contractions was negatively correlated to mixed muscle CFPE index across all participants.

*High CFPE vs Low CFPE group:* Prior to the intervention, there were no significant differences in plasma IL-6 concentrations in the High compared to the Low group. In the High group, plasma IL-6 concentration was significantly increased at 6h ( $1.9 \pm 0.3$  pg/mL,  $p < 0.05$ ) and 24h ( $1.8 \pm 0.2$  pg/mL,  $p < 0.05$ ) but was not significantly different at 72h ( $1.2 \pm 0.2$  pg/mL) as compared to Pre ( $1.2 \pm 0.2$  pg/mL). In the Low group, plasma IL-6 concentration was significantly increased at 6h ( $2.7 \pm 0.2$  pg/mL,  $p < 0.05$ ) and 24h ( $1.7 \pm 0.1$  pg/mL,  $p < 0.05$ ) but

was not significantly different at 72h ( $1.3 \pm 0.1$  pg/mL) as compared to Pre ( $1.2 \pm 0.2$  pg/mL). In comparing the IL-6 to eccentric exercise in the High CFPE as compared to the Low CFPE group, we observed that there was a greater change in plasma IL-6 concentrations from Pre to 6h in the Low group as compared to the High group (Supplementary Figure 2B;  $p < 0.05$ ).

## **DISCUSSION:**

In the present study, we observed that there was an enhanced expansion and activation of the SC pool in individuals with high as compared to low capacity for muscle perfusion following eccentric contractions. Therefore muscle fibre capillarization may be a critical factor for the activation and expansion of the SC pool in response to muscle damage in humans. Furthermore, individuals with a higher capacity for muscle perfusion experienced a more rapid force recovery following muscle damage as compared to the group with a lower capacity for muscle perfusion.

SC are indispensable for the repair and/or regeneration of damaged muscle in rodents (Lepper *et al.*, 2011; McCarthy *et al.*, 2011; Sambasivan *et al.*, 2011). In humans, a single bout of high-velocity eccentric contractions results in increased plasma creatine kinase, reduced force production and myofibrillar ultrastructural damage (Beaton *et al.*, 2002; Clarkson & Hubal, 2002; Paulsen *et al.*, 2012). Consequently, eccentric contractions are an effective tool for expansion of the muscle SC pool (Cramer *et al.*, 2004; Dreyer *et al.*, 2006; McKay *et al.*, 2008; McKay *et al.*, 2009; Cermak *et al.*, 2013) though the degree of expansion is dependent on many factors (Snijders *et al.*, 2015). However, the specific factors that determine the degree of activation and expansion of the SC pool are not well understood. In agreement with previous literature, we report that there is an expansion in the SC pool (as determined by total Pax7<sup>+</sup> cells/100 myofibre) and an increase in SC pool activation (as determined by MyoD<sup>+</sup>/Pax7<sup>+</sup> cells/100 myofibre) in the

days following a single bout of eccentric contractions. To better understand factors that determine the degree of activation and expansion of the SC pool we examined whether muscle fibre capillarization may be a determining factor following an acute bout of eccentric contractions in young men. Skeletal muscle capillarization and perfusion is necessary for the delivery of oxygen, growth factors and macronutrients to muscle fibres and resident cell populations alike. We and others have previously already reported an anatomical relationship between muscle SC and capillaries (Christov *et al.*, 2007; Nederveen *et al.*, 2016; Nederveen *et al.*, 2017), suggesting that the proximity of SC to their nearest capillary may be a determining factor in their activation status (Christov *et al.*, 2007; Nederveen *et al.*, 2016). In the present study, there was a positive correlation between the expansion of the total SC pool 24h post-eccentric exercise and mixed muscle CFPE, an index of muscle perfusion, suggesting that the greatest SC pool size expansion was experienced by subjects with the highest capacity for muscle fibre perfusion. When participants were retrospectively divided based on their mixed muscle CFPE index into a High CFPE and Low CFPE group, we observed that there was a greater expansion of the total Pax7<sup>+</sup> SC pool in the group with high CFPE (High; CFPE index  $7.6 \pm 1.0$  capillaries  $\cdot 1000 \mu\text{m}^{-1}$ ) as compared to low CFPE (Low; CFPE index  $5.2 \pm 0.5$  capillaries  $\cdot 1000 \mu\text{m}^{-1}$ ). This observation was made 6h ( $\sim 48\%$  vs.  $\sim 1\%$  Pax7<sup>+</sup> cells/100 myofibre, respectively) and 24h ( $\sim 73\%$  vs.  $\sim 10\%$  Pax7<sup>+</sup> cells/100 myofibre, respectively) post-eccentric contractions. Work by Christov and colleagues (2007) supports these findings as they observed a correlation between fibre capillarization and SC content in human deltoid muscle in the resting state, regardless of muscle fibre type. Furthermore, we observed that the High group had a greater activation of the SC pool at 6h ( $\sim 750\%$  vs.  $\sim 450\%$  MyoD<sup>+</sup>/Pax7<sup>+</sup> cells/100 myofibre, respectively) and 72h ( $\sim 750\%$  vs.  $\sim 300\% \pm$  MyoD<sup>+</sup>/Pax7<sup>+</sup> cells/100 myofibre,

respectively). Interestingly, although the degree of muscle damage was similar between groups (assessed by increases creatine kinase activity, reduction in force production), we observed that the force production returned to baseline again at 96h post-exercise recovery in the High group, whereas this was not the case in the Low group. Together with the greater activation and expansion of the SC pool size observed in the High group during post-exercise recovery these data indicate that individuals with a high CFPE index have an accelerated muscle fibre recovery response following an acute bout of damaging exercise.

In the present study we observed that participants in the High group had a significantly greater percentage of type I muscle fibres as compared with the Low group (~56% vs ~34% Type I fibres, respectively). Type I muscle fibres are more oxidative, associated with more capillaries and/or are perfused to a greater degree than their type II counterparts. Considering that muscle fibre capillaries are shared amongst a mosaic of fibre types in humans, a greater percentage of type I muscle fibres may result in enhanced perfusion of neighboring type II muscle fibres. A greater association with shared muscle fibre capillaries amongst the muscle fibre type mosaic may contribute to not only a greater type II muscle fibre perfusion, but also contribute to a closer proximity between type II Pax7<sup>+</sup> SC and the nearest capillary observed in the High group. Consistent with this notion, we observed a negative correlation between type II muscle fibre CFPE index and type II Pax7<sup>+</sup> SC distance to their nearest capillary. Considering that SC distance to nearest capillary in type II muscle fibres was negatively correlated with a greater change in type II Pax7<sup>+</sup> cells/100 myofibre from Pre to 24h following eccentric exercise, we propose that the link between a greater SC activation/expansion in response to muscle fibre damage may be the reduced spatial proximity to microvascular capillaries. In line with this, we have previously observed that type II SC are located at a further distance from capillaries in older

men as compared to their young counterparts (Nederveen JCSM). Older men typically exhibit an impaired expansion and/or activation response to exercise (McKay *et al.*, 2012; Snijders *et al.*, 2014), as well as lower basal SC content (Verdijk 2007) concomitant with a loss of muscle capillarization (Proctor 2005). Taken together, these data support a relationship between muscle capillarization and functional SC in humans.

We also observed that enhanced capacity for muscle perfusion (i.e., muscle CFPE) or a reduction in the distance of a SC to its nearest capillary was associated with an enhanced activation and expansion of the SC pool in response to eccentric contraction-induced muscle fibre damage in mixed, type I and type II muscle fibres. Previously, we reported that following resistance training, there was an increase in muscle capillarization and also an enhanced activation of SC in response to an acute bout of resistance exercise (Nederveen *et al.*, 2017). Taken together, this suggests that muscle capillarization and the ability of the SC pool to activate and expand following exercise/damage are closely linked. Indeed, it is now well established that activated SC are found at closer proximity to capillaries than their quiescent counterparts (Chazaud *et al.*, 2003; Christov *et al.*, 2007; Nederveen *et al.*, 2016). However, the specific cues for induction of the myogenic program in response to muscle fibre damage remain to be elucidated. The process of SC activation, proliferation and/or differentiation is regulated by a multitude of cytokines and growth factors (e.g., IL-6, IGF-1, myostatin, HGF) (Kadi *et al.*, 2005). Considering that CFPE index is regarded as a proxy measure of microvascular perfusion (Hepple & Mathieu-Costello, 2001; Weber *et al.*, 2006), variations in CPFE index could modify delivery of circulating nutrients and/or growth factors and presumably change the local environment of a SC post exercise/damage (Conboy *et al.*, 2005; Brack & Rando, 2007). In this capacity, few growth factors have been as extensively investigated as the cytokine IL-6, a well

characterized member of the interleukin family. IL-6 is known to respond to various forms of exercise (Pedersen & Febbraio, 2008), but importantly known to play a role in SC function (Toth *et al.*, 2011; McKay *et al.*, 2013). Furthermore, elevations of IL-6 concentration has been shown to be associated specifically with SC proliferation in response to muscle fibre injury (Pedersen & Febbraio, 2008; Toth *et al.*, 2011; McKay *et al.*, 2013). In the current study, we observed that individuals with a lower mixed muscle CFPE index had a greater increase in plasma concentration of IL-6 from Pre to 6h following eccentric exercise. In line with this, we observe that the Low as compared to the High group had a greater change in circulating IL-6 from the Pre to 6h (increase of ~163% vs. ~66%, respectively). Interestingly, the greater plasma concentration of IL-6 observed in the Low group occurred simultaneously with a lesser activation of muscle SC (i.e., MyoD+/Pax7+ cells/100 myofibre) over this same time period. Diminished activation of SC in the Low group despite an elevated systemic plasma IL-6 response in comparison to the High group suggests that there may be other mechanisms that regulate the impact of systemic IL-6 concentration upon SC activation and/or proliferation. Previous work has established that the presence of IL-6 can reduce endothelial signalling in some physiological situations (Yuen *et al.*, 2009). Increased plasma IL-6 concentrations may have implications for an increased local SC niche concentration, and may therefore interfere with the observed cellular cross-talk between SC and endothelial cells (Chazaud *et al.*, 2003; Ochoa *et al.*, 2007). Previous work suggests that IL-6 is produced by various resident cell types such as macrophages (Zhang *et al.*, 2013), fibroblasts (Joe *et al.*, 2010) or endothelial cells (Sironi *et al.*, 1989; Yan *et al.*, 1995), the exercising muscle (Steensberg *et al.*, 2000; Pedersen & Febbraio, 2008) as well as SC themselves (Kami & Senba, 2002). Future work should continue to address the paracrine and autocrine functions of increased IL-6 within the local SC niche.

Given the positive relationship between muscle capillarization and the activation and expansion of the SC pool we conclude that the SC response is modulated by cross-talk with endothelial cells within the microvasculature, exposure to circulating signals, or a combination of both. In the future, attention should be focused on study populations who are compromised with respect to muscle capillarization (Coggan *et al.*, 1992; Proctor *et al.*, 1995), and/or impaired SC content at rest and in response to exercise (McKay *et al.*, 2012; Snijders *et al.*, 2014) such as in the elderly. These future studies may provide insight into whether the blunted post-exercise SC response in elderly individuals can be improved by increasing muscle fibre capillarization. In conclusion, the present study shows that skeletal muscle fibre capillarization is a significant factor for muscle SC activation and pool size expansion, thereby accelerating the muscle repair response following muscle damage in healthy young men.



## Figure Legends

Figure 1

**Fig. 1** Fibre type specific satellite cell staining with muscle capillaries. (A) Representative image of a MHCI/laminin/CD31/Pax7/DAPI stain of a muscle cross section. Channel views of (B) Merge (C) Pax7/DAPI (D) Pax7/CD31 (E) Pax7/MHCI/Laminin (F) Characterization of the expansion of the total mixed muscle satellite cell (SC) pool before and after 6h, 24h, 72h and 96h following eccentric contractions in the group with a High capillary to fibre exchange (CFPE) index and the group with Low CFPE index. \*, Significantly different compared with Pre ( $p < 0.05$ ), bar indicates that effect of time is present for both groups. #; indicates a significantly greater increase with time High vs Low group ( $p < 0.05$ ). Data are expressed as mean  $\pm$  sem. Relationship between the expansion of the total SC pool and mixed muscle CFPE following (G)  $\Delta 24h$  post-eccentric exercise ( $r = 0.39$ ,  $p < 0.05$ ) and (H)  $\Delta 72h$ -post exercise ( $r = 0.15$ ,  $p > 0.05$ ) across all participants.

Figure 2

**Fig. 2** Mixed muscle staining of satellite cell (SC) activation with muscle capillaries. (A) Representative image of a CD31/Pax7/MyoD/DAPI stain of a muscle cross section). Channel view of (B) Pax7/DAPI (C) MyoD/DAPI (D) Pax7/CD31 (E) MyoD/CD31 (F) Characterization of the activation status of the SC pool before and after 6h, 24h, 72h and 96h following eccentric contractions in the group with a High capillary to fibre exchange (CFPE) index and the group with Low CFPE index. \*, Significantly different compared with Pre ( $p < 0.05$ ), bar indicates that effect of time is present for both High and Low group. #; indicates a significantly greater increase with time High vs Low group ( $p < 0.05$ ). Data are expressed as mean  $\pm$  sem. Relationship between the activation of the SC pool ( $\Delta \text{MyoD}^+/\text{Pax7}^+$  cells) and mixed muscle CFPE following (G)  $\Delta 6h$  post-eccentric exercise ( $r = 0.40$ ,  $p < 0.05$ ) and (H)  $\Delta 72h$ -post exercise ( $r = 0.37$ ,  $p < 0.05$ ) across all participants.

Figure 3

**Fig. 3** Characterization of the expansion of type II fibre-associated satellite cell (SC) pool following eccentric contractions before and after 6h, 24h, 72h and 96h following eccentric contractions in the group with a High capillary to fibre exchange (CFPE) index and the group with Low CFPE index. (A) \*, Significantly different compared with Pre ( $p < 0.05$ ), bar indicates that effect of time is present for both groups. #; indicates a significantly greater increase with time High vs Low group ( $p < 0.05$ ). Data are expressed as mean  $\pm$  sem. Relationship between the expansion of the type II SC pool and type II CFPE following (B)  $\Delta 6h$  post-eccentric exercise ( $r = 0.45$ ,  $p < 0.05$ ) and (C)  $\Delta 24h$ -post exercise ( $r = 0.42$ ,  $p < 0.05$ ) across all participants.

Figure 4

**Fig. 4** Relationship between the expansion of the satellite cell (SC) pool from Pre to 24h post-eccentric exercise in a fibre type specific manner and fibre type specific CFPE for (A) type I-associated SC ( $r = 0.05$ ,  $p = 0.79$ ) and (B) type II-associated SC ( $r = -0.39$ ,  $p < 0.05$ ) across all participants. Relationship between fibre type specific Capillary to fibre exchange (CFPE) index and distance of Pax7<sup>+</sup> SC to nearest capillary following eccentric exercise prior to eccentric damage for (C) type I-associated SC ( $r = -0.22$ ,  $p = 0.15$ ) and (D) type II-associated SC ( $r = -0.51$ ,  $p < 0.05$ ) across all participants.

#### Supplementary Figures

##### Supplementary Figure 1

**Sup.1** (A) Characterization of the force production (nm) response following eccentric contractions in the overall group. \*; Significantly different compared with Pre ( $p < 0.05$ ) (B) in the group with a high capillary to fibre exchange (CFPE) index and the group with low CFPE index. \*; Significantly different compared with Pre in the Low CFPE group ( $p < 0.05$ ), bar indicates that effect of time is present for both groups. (C) Characterization of the creatine kinase activity (CKA; mU/mL) response following eccentric contractions in the overall group \*; Significantly different compared with Pre ( $p < 0.05$ ) (D) in the group with a high capillary to fibre exchange (CFPE) index and the group with low CFPE index. . Bar indicates significantly different compared with Pre ( $p < 0.05$ ), present for both group

##### Supplementary Figure 2

**Sup.2** (A) Relationship between the change IL-6 concentrations (expressed as a percentage) from Pre to 6h post-eccentric exercise and mixed muscle CFPE ( $r = -0.42$ ,  $p < 0.05$ ) across all participants. (B) Characterization of the IL-6 (pg/mL) response following eccentric contractions in the overall group \*; significantly different compared with Pre ( $p < 0.05$ ) (C) Relationship between the change IL-6 concentrations (expressed as a percentage) from Pre to 72h post-eccentric exercise and mixed muscle CFPE ( $r = -0.43$ ,  $p < 0.05$ ) across all participants. (D) Characterization of the IL-6 response (expressed as a percentage) in the group with a high capillary to fibre exchange (CFPE) index and the group with Low CFPE index. Bar indicates significantly different compared with Pre ( $p < 0.05$ ), present for both group. #; indicates a significantly greater change with time High as compared to the Low CFPE group ( $p < 0.05$ ).

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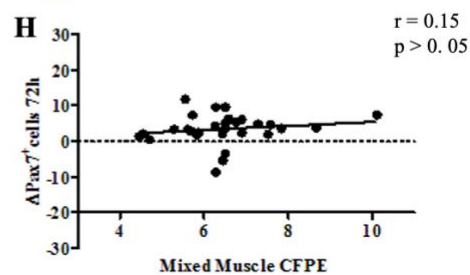
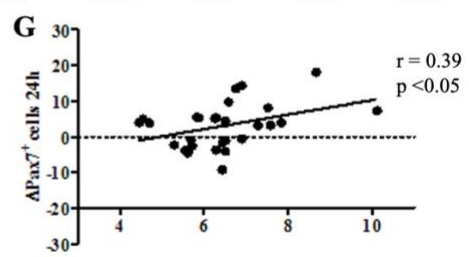
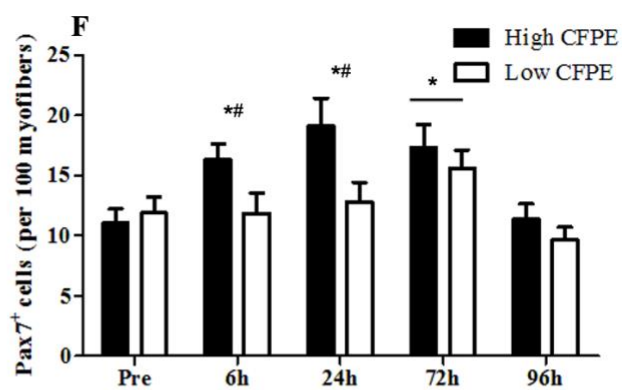
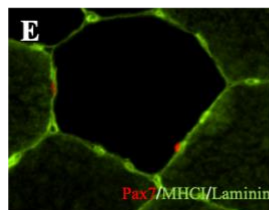
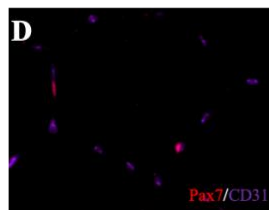
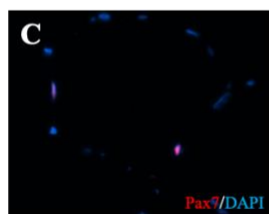
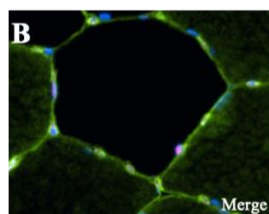
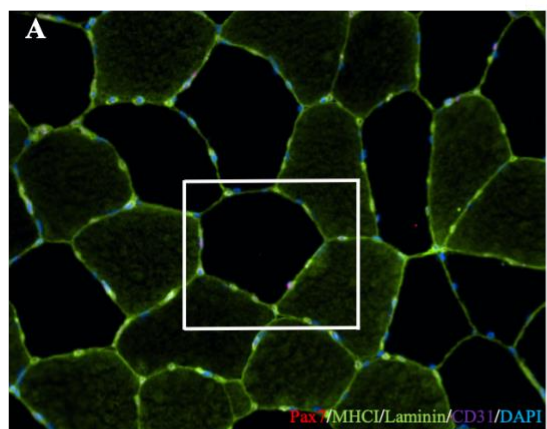
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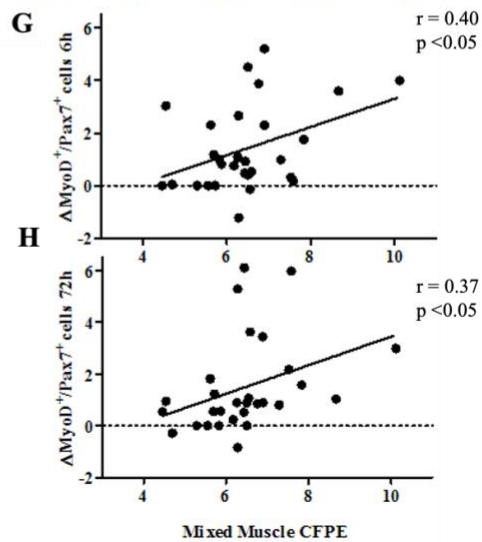
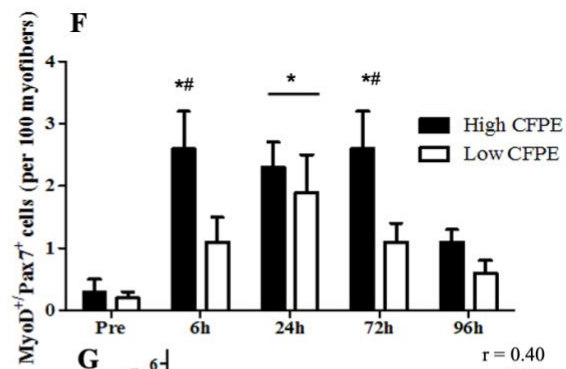
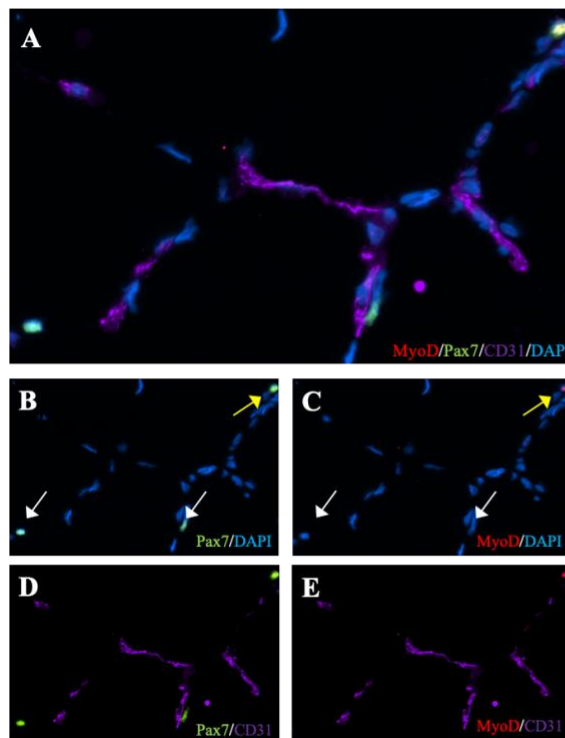
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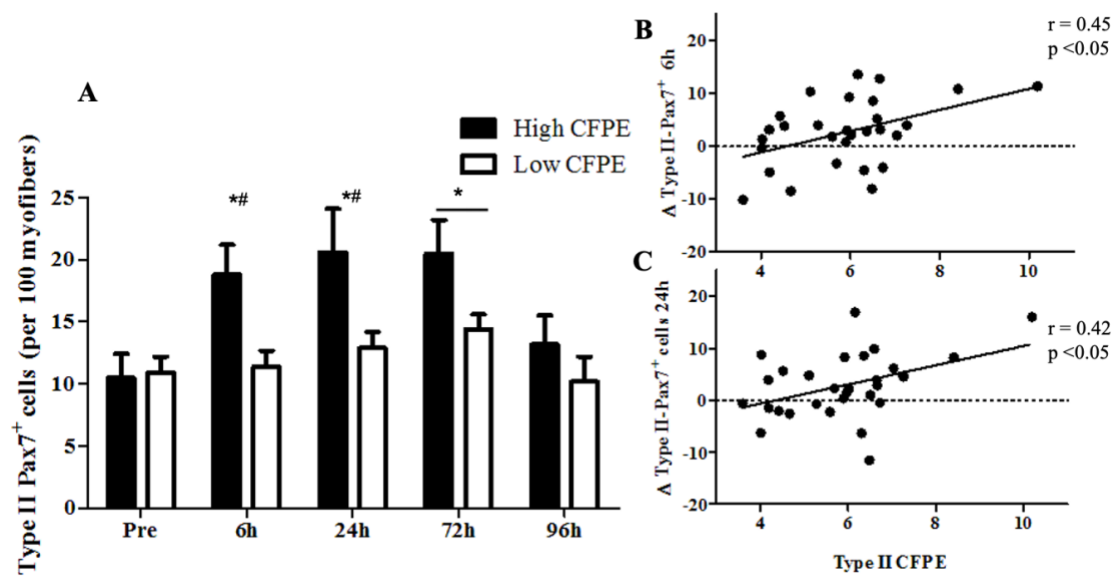
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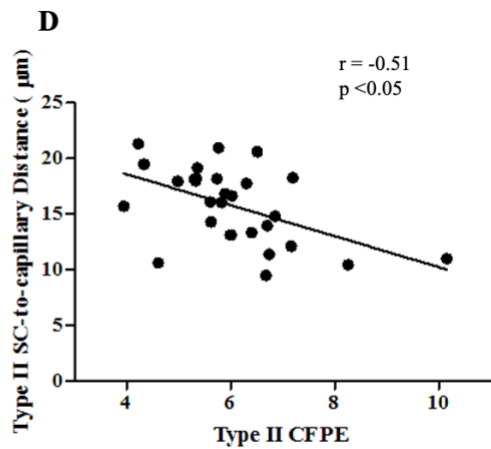
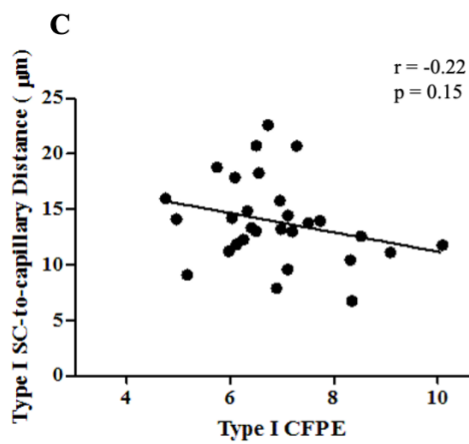
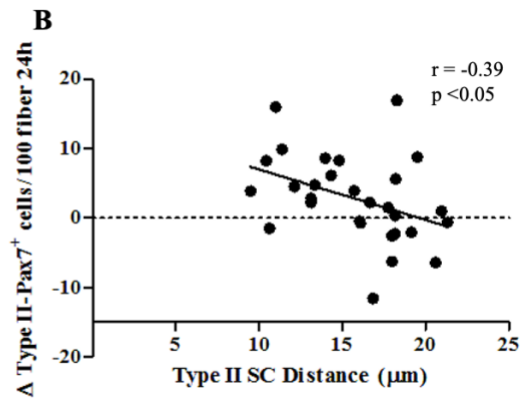
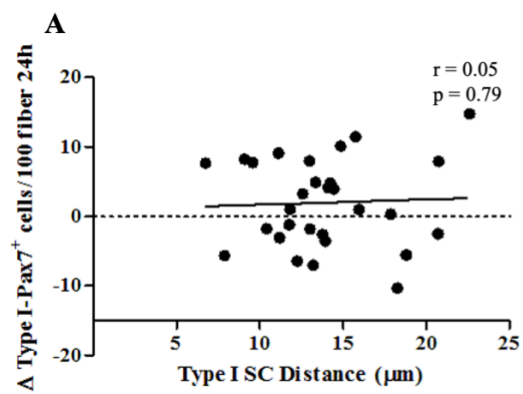
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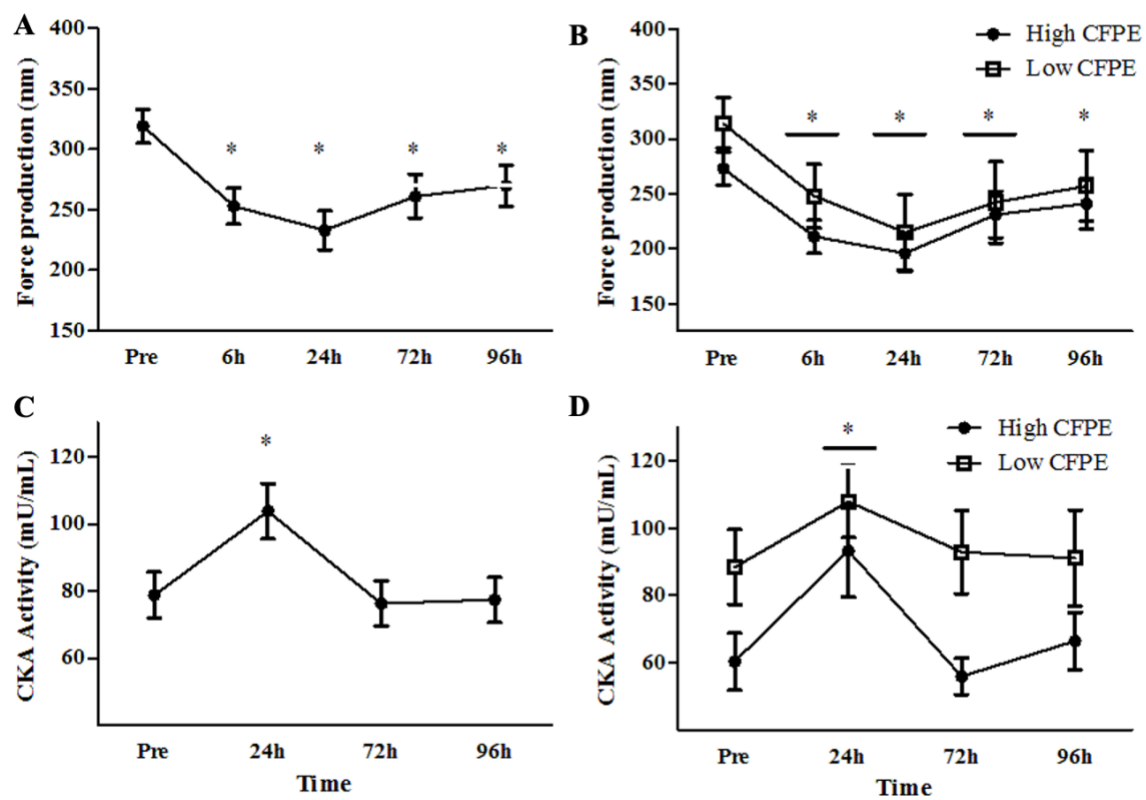








Supplementary  
Figure 1



Supplementary  
Figure 2

